CELL CONTACT MEDIATED DIFFERENTIATION IN *DICTYOSTELIUM*

By

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Major Department: Zoology

Upon spore germination in the cellular slime mold *Dictyostelium discoideum* amoeboid cells are liberated which proceed to engulf bacteria as foodstuff and undergo binary fission. After large numbers of the myxamoebae are produced aggregation of the cells in response to a chemotactic substance, cyclic AMP, results in the formation of multicellular pseudoplasmodia. Differentiation of two cell types, the prestalks and the prespores, occurs as a result of cell association. Eventually each aggregate forms a mature sorocarp composed of a slender stalk bearing a mass of spores. Major biochemical and ultrastructural changes occur at the cell surfaces following cell association. Cyclic AMP which effected the formation of certain plasma membrane particles during aggregation and Con A which binds to specific membrane sites were used to clarify the nature of the cellular interactions. Cyclic AMP induced major increases in the sizes of the plasma membrane particles among isolated aggregating cells but prespore vacuoles indicative of prespore differentiation did not appear. Aggregating

streams exposed to Con A failed to synthesize large plasma membrane particles and prespore differentiation was inhibited. A few cells bearing large particles were found among the mechanically disrupted aggregates. Differentiation did not occur among these cells suggesting that a continuous period of cell association and interaction is essential. Prespore cells exposed to Con A just prior to differentiation into mature spores formed aberrant spore walls and irregularly shaped spores. The inhibitory effect of Con A on prespore and mature spore differentiation is discussed. The large plasma membrane particles were considered to be the components through which cell interactions are mediated resulting in differentiation.

INTRODUCTION

The existence of cellular interactions as a factor in cytodifferentiation has long been recognized. The classical experiment of Spemann and Mangold (1924) demonstrated that the induction of neural structures in amphibian embryos depended upon an interaction between the ectoderm and chorda mesoderm. The concept of inductive interactions was exploited and a similar phenomenon was shown by Spemann (1901) to occur in the induction of a lens by the eyecup in *Rana fusca*. Subsequently numerous other examples of a similar nature were shown to exist among developing organisms.

Eventually the question arose as to the nature of the inductive processes and the degree of contact necessary between two tissue types to effect cytodifferentiation. This led in one instance to the insertion of a semi-permeable barrier between the inducing optic vesicle of a chick and the overlying ectoderm (McKeehan, 1951). The failure of lens induction under these circumstances emphasized the necessity for some form of macromolecular communication during the inductive period.

A series of experiments were conducted to determine the degree of association required in inductive interactions. By the use of millipore filters separating mesenchymal components of various types of rudiments from appropriate epithelium it was demonstrated that induction of the epithelium was still possible (Grobstein, 1961, 1963; Golosow and Grobstein, 1962). The inductions were attributed primarily to the transmission of large molecular weight substances through the

20 μ thick filters. However, the average pore diameter of the filter was 5,000 Å which could not exclude certain cytoplasmic processes.

A unique type of cellular interaction is recognized in the differentiation of skeletal muscle cells. The primordial muscle cells or myoblasts following a period of proliferation fuse to form multinucleated myotubes. The myotubes subsequently differentiate exhibiting cross-striations characteristic of skeletal muscle in the myofibrils (Konigsberg, 1963).

Among the systems employed in studies of cellular interactions the cellular slime mold *Dictyostelium discoideum* offers certain advantages. The germination of mature spores provides a population of vegetative myxamoebae which feed upon appropriate bacteria and undergo binary fission (Figures 1a, 1b). The myxamoebae of the vegetative stage exist independently until they aggregate in response to the chemotactic agent acrasin (cyclic AMP) which is initially secreted by individual or small groups of cells (Bonner, 1947; Konijn *et al.*, 1967)(Figures 1c, 1d). Ultrastructurally the myxamoebae appear to be identical. However, by late aggregation two cell types may be distinguished in the pseudoplasmodium (Figure 1e). The cells which become prespore cells contain prominent organelles named prespore vacuoles (PV) while the other cell type, the prestalk, is devoid of these 6,000 Å structures (Hohl and Hamamoto, 1969) (Figure 2). The posterior two thirds of the migrating pseudoplasmodium is composed of prespore cells while the anterior prestalk cells occupy the remainder of the cell mass (Bonner, 1952). By further morphogenetic movements each pseudoplasmodium forms a mature fruiting body consisting of a slender stalk bearing a mass of mature spores at the apex (Figure 1a).

Figure 1. Life cycle of the cellular slime mold *Dictyostelium discoideum*-1H.

a. mature sorocarp (inset: group of spores)
b. vegetative myxamoebae
c. aggregating myxamoebae
d. aggregating center with streams
e. late aggregate
f. migrating pseudoplasmodium
g. preculminate
h. early culmination
i. culmination stage just prior to mature spore differentiation
j. culmination stage following mature spore formation

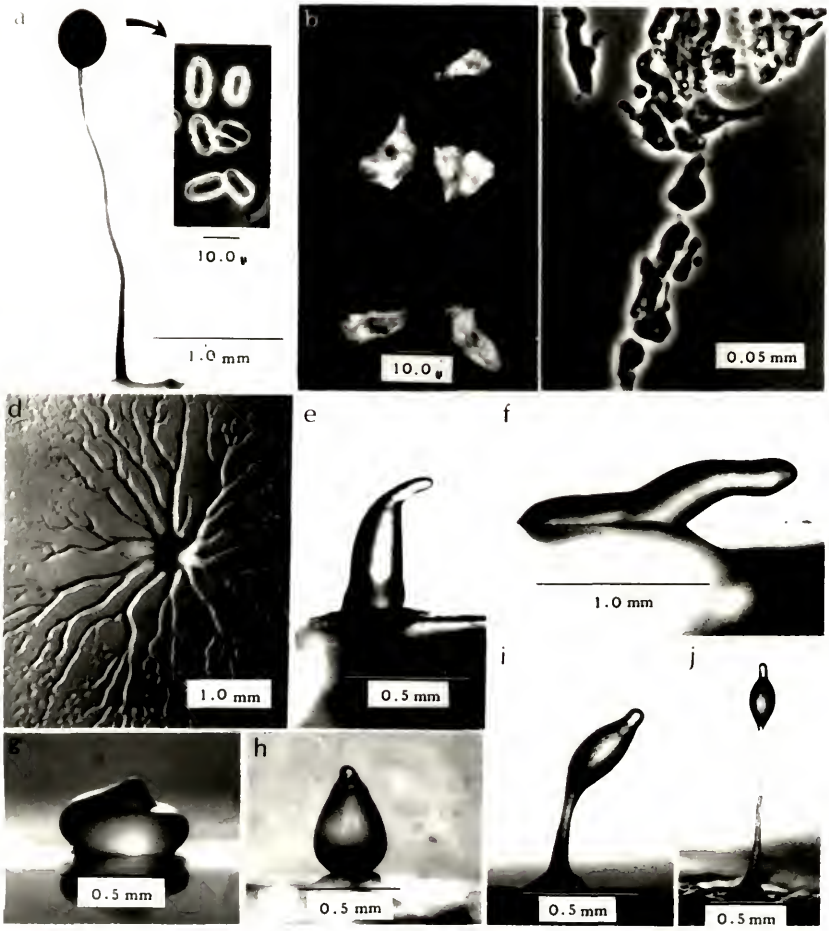
a
10.0
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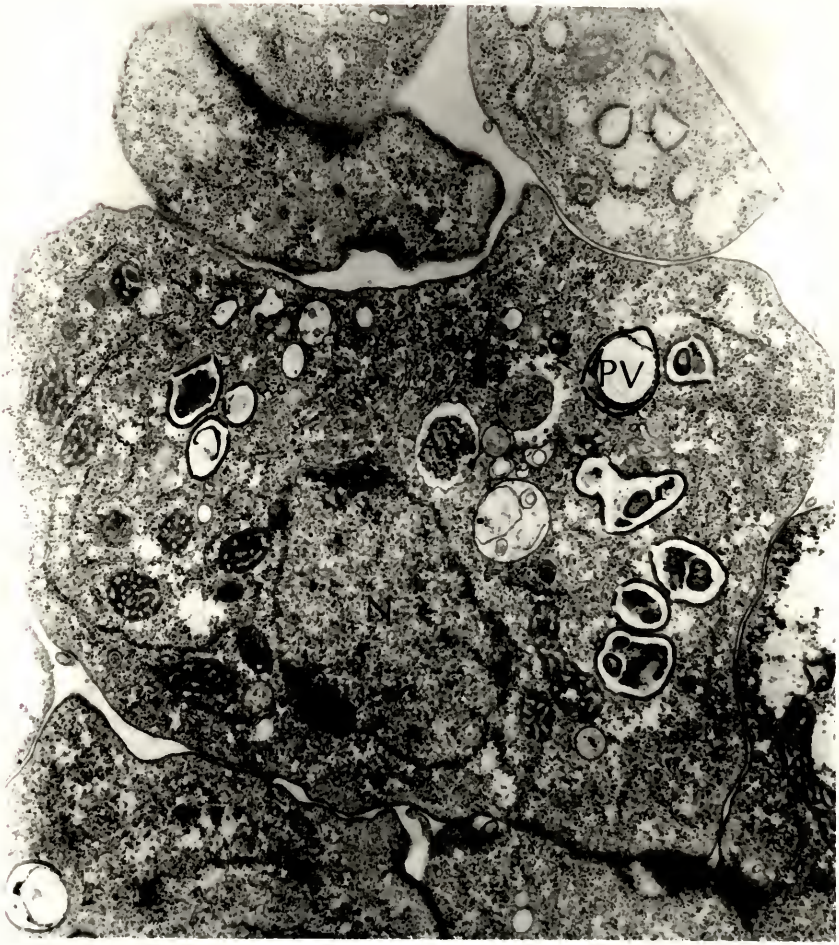
Figure 2. Prespore cell in an early culminating stage, X19,566.

AV - autophagic vacuole
M - mitochondrion
N - nucleus
PV - prespore vacuole


PV
N

The discrete groups of prestalk and prespore cells in the migrating pseudoplasmodium may be isolated to determine their capacity for cell redifferentiation and the regulation of proportions. Raper (1941) noted that both isolated prestalk and prespore cell groups could redifferentiate the missing cell types and form normal fruiting bodies. Gregg and Badman (1970) observed that prestalk and prespore cell group isolates were capable of synthesizing or losing PV respectively, in the course of redifferentiating. If aggregating myxamoebae or prestalk and prespore cells were isolated not in groups but as single cells, neither differentiation nor redifferentiation occurred (Gregg, 1971). Evidence is also available which suggests that cell association regulates the synthesis of certain enzymes in *Dictyostelium*. Disaggregated cells maintained in isolation were observed to cease production of the enzymes (Loomis, 1970; Newell *et al.*, 1971).

The necessity of cell contact in the differentiation of *D. discoideum* myxamoebae suggests that the plasma membranes must be intimately involved. Ample evidence exists that the plasma membranes undergo changes during the transition of the myxamoebae from unicellular state to a multicellular existence. Shaffer (1958) first noted that the myxamoebae upon aggregation became adhesive in response to the effect of acrasin. Simultaneously new surface antigens appear (Gregg, 1956) which may be involved in the maintenance of contacts between apposing cells (Beug *et al.*, 1970). Freeze-fracture studies revealed that particulate structures in the plasma membranes increased 1.7X in average diameter from the vegetative stage to the prespore cells in migrating pseudoplasmodia (Figures 3, 4) (Aldrich and Gregg, 1973). Plasma membranes

Figure 3. Freeze-fractured vegetative myxamoebae plasma membrane exhibiting particles averaging 60 Å in diameter, X300,000.

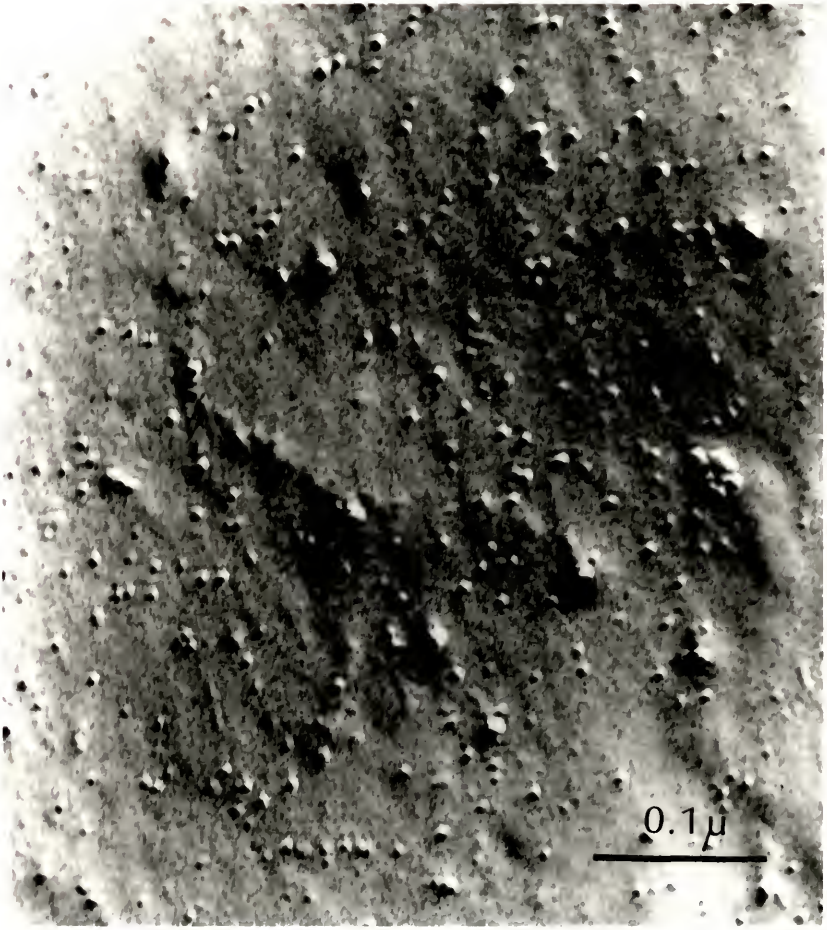

0.1μ

Figure 4. Freeze-fractured late aggregate prespore cell plasma membrane exhibiting particles averaging 106 Å in diameter, X300,000.


0.1μ

of many species are characterized by the presence of particulate structures. In red blood cells the membrane particles are considered to be composed of glycoproteins which interact with intramembranous proteins or lipids. The glycoproteins which extend through the membrane expose carbohydrate moieties at the cell surface (Marchesi *et al.*, 1972). Indeed carbohydrate is bound to all the major surface proteins (Steck, 1974). The large plasma membrane particles in *Dictyostelium* are considered to project through the membrane and be exposed at the cell surface (Aldrich and Gregg, 1973). Singer and Nicolson (1972) suggest that contacts between the particulate structures of apposing cells may result in the transmission of stimuli into the cytoplasm thereby initiating cell differentiation.

Dictyostelium cells may be quickly agglutinated by Concanavalin A (Con A) (Weeks, 1973). Con A, a phytagglutinin isolated from the jack bean, *Canavalia ensiformis*, is known to bind reversibly to oligosaccharides containing terminal α-D-glucopyranosyl, α-D-mannopyranosyl and sterically related sugar residues. Thus, Con A can bind to glycoproteins and possibly glycolipids on the cell surfaces of a variety of cell types (Edelman, 1973).

It is clear that differentiation in *Dictyostelium* normally depends upon an association between the cells beginning at the aggregation phase. The ultrastructural changes which appear in the plasma membranes following aggregation may provide the morphological and functional basis upon which cellular interactions depend. Cyclic AMP and Con A, both of which bind to specific cell sites on the plasma membranes, were utilized in the course of this study. The effects of these substances on development

may yield information on the origin and nature of the interactions which affect the transformation of the cells.

METHODS AND MATERIALS

Cellular Slime Molds

Wild-type *Dictyostelium discoideum*-1H was used throughout this investigation. The myxamoebae were cultured at 22-23°C on nutrient agar plates using *Escherichia coli* as the bacterial associate (Bonner, 1947).

Harvesting of *D. discoideum* cells

a. Vegetative myxamoebae harvested from 17-22-hour cultures were washed free of bacteria 3X in 0.013 M phosphate buffer (PB) at pH 7 by low speed centrifugation in a clinical centrifuge. The cells to be used in subsequent experiments were concentrated at 1×10^6/ml as determined by counts made with a hemocytometer.

b. Aggregating stages were prepared from 24-hour cultures of vegetative myxamoebae. The washed cells were distributed on 2% non-nutrient (NN) agar buffered at pH 7. The myxamoebae formed aggregating streams within 11 hours.

c. Early culminating stage pseudoplasmodia were collected as individuals by removing 5 mm cylinders of agar each bearing a slime mold. This procedure utilized a sharp metal tube connected to a rubber tube mouth aspirator to apply the small vacuum necessary in retaining the agar plug as it was transferred to another culture dish containing NN agar.

Thin-section Electron Microscopy

Vegetative and aggregation stage myxamoebae were fixed in 2% glutaraldehyde (Ladd Research Industries) containing standard salt solution (SS) (Bonner, 1947) at 4°C for 15-20 minutes. The fixative was adjusted to pH 7 just prior to use by stirring with barium carbonate and filtration through Whatman #50 paper. The cells were post-fixed in 1% osmium tetroxide containing SS for 20 minutes and stained with 0.5% uranyl acetate at pH 3.9 for 20 minutes (Terzakis, 1968). Dehydration was effected in ethanol and acetone followed by embedding in Epon 812 (Ladd Research Industries). Thin-sections were post-stained in 2% aqueous uranyl acetate for 15 minutes and Reynolds (1963) lead citrate for 4 minutes. The sections were examined and photographed by an Hitachi HU 11-E electron microscope at 75 KV.

Migrating pseudoplasmodia, culminating stages and mature sorocarps were fixed in 2% glutaraldehyde containing 0.1 M cacodylate buffer at pH 7 at 4°C for 1 hour. The cells were post-fixed in cacodylate-buffered 1% osmium tetroxide at 4°C for 2 hours. The remainder of the preparation was identical to that described for the vegetative and aggregation stage myxamoebae.

Freeze-fracturing

Myxamoebae composing aggregating streams and late aggregation stages were fixed for 30 minutes at 4°C in 2% glutaraldehyde in SS which had been adjusted to pH 7. The cells were washed 3X in SS for 10 minutes/wash. Following exposure to 20% glycerol for 3-24 hours the cells were mounted on gold-nickel discs, frozen in Freon 22 and stored in liquid nitrogen. The freeze-fracturing technique and production of

carbon-platinum replicas were essentially identical to those used by Moor and Mühlethaler (1963). A Balzers BA 360 M apparatus was used to produce 13 replicas which were subsequently photographed at 150,000X. The plasma membrane particle sizes were derived by analyzing 27 photographic negatives in the manner described by Goodenough and Staehelin (1971) and Gregg and Nesom (1973).

Purification of Concanavalin A

Con A (Calbiochem) was dissolved in 0.001 M phosphate buffer with the addition of 0.086 M NaCl, 0.0027 M KCl, 0.0001 M $CaCl_2$ and 0.0001 M $MnCl_2$. Centrifugation at low speed in a clinical centrifuge for 5 minutes was necessary to remove any large aggregates. The supernatant was passed through a 2.2 X 40 cm G-75 Sephadex column which had been equilibrated with the Con A buffer (CAB). Bonding of the Con A to the Sephadex enabled the column to be eluted with CAB to eliminate contaminating proteins. The Con A was then recovered by eluting the column with CAB containing 0.1 M α-D-glucose or α-D-mannose. Two-milliliter fractions were collected for spectrophotometric determinations at 280 nm. The fractions containing Con A were dialyzed against CAB to remove excess sugar. Following dialysis the Con A was lyophilized and subsequently diluted to the concentrations required for a particular experiment.

RESULTS

Vegetative Myxamoebae

a. Inhibition of morphogenesis and differentiation by Con A

NN agar plates were prepared containing Con A ranging in concentration from 50-800 µg/ml agar. Control plates consisted of NN agar containing only CAB. Using a micropipette 200 µl of 22-hour vegetative myxamoebae cultures at a concentration of 1×10^6 cells/ml were delivered to the plates. The cells were uniformly distributed over the agar surface with a glass rod.

Observations over a period of 75 hours disclosed that Con A causes a delay in the appearance of aggregates. The amount of delay depends upon the dosage which also affects the number of fruiting bodies which ultimately appear (Table 1).

b. Cyclic AMP effects on prespore cell differentiation

Vegetative myxamoebae from 17-hour cultures following the SS wash to free them from bacteria were further washed for approximately 5 minutes in 0.001 M cyclic AMP in SS. The cells were then isolated as individuals on NN agar containing 0.001 M cyclic AMP for 3 hours. At the end of this period the cells were fixed and prepared for thin-sectioning. Subsequent examination with the electron microscope revealed that the cells had not synthesized PV typically found in prespore cells.

TABLE 1

Inhibition of morphogenesis in vegetative myxamoebae exposed to agar containing Con A*

Con A solutions in µg/ml agar	Hours at which various degrees of development were observed: Aggregates	First appearance of mature sorocarps	Maximum no. of mature sorocarps
0	9	26	31 (control)
50	20	26	50 (100)**
100	20	44	75 (100)
200	20	44	75 (100)
300	26	44	75 (90)
400	26	44	75 (80)
500	26	44	75 (70)
800	26	44	75 (20)

*The cultures were examined to determine the stage of development following 9, 20, 26, 31, 44, 50, and 75 hours of exposure to Con A.

**Percentage of mature sorocarps compared to control.

Aggregating Streams

a. Cyclic AMP effects on prespore cell differentiation

The aggregating streams were washed once in SS containing 0.001 M cyclic AMP and dispersed as individuals on NN agar containing 0.001 M cyclic AMP for a period of 6 hours. The control streams washed only in SS were dispensed as small dense groups of myxamoebae on NN agar for a similar period.

The dispersed cyclic AMP treated cells examined in thin-section did not exhibit PV formation. However, cyclic AMP promotes adhesiveness between the cells which resulted in a number of small aggregates. The cells composing the aggregates were found to have synthesized PV and were considered to have differentiated into prespore cells. The control streams within the 6-hour period had formed late aggregates and migrating pseudoplasmodia (Figures 1e, 1f) which emphasized that sufficient developmental time had elapsed for prestalk and prespore cells to differentiate.

b. Cyclic AMP effects on plasma membrane structure

The aggregating streams exposed to cyclic AMP as described in "a" above were subjected to freeze-fracturing.

Freeze-fracturing of the cyclic AMP treated cells revealed that plasma membrane particles had been synthesized which averaged 97 Å (Table 2). The control preparation had formed late aggregates and migrating pseudoplasmodia. The prespore cells composing these stages exhibited plasma membrane particles averaging 101 Å (Table 2).

c. Con A effects on prespore cell differentiation

The streams from several aggregates were collected carefully with a hair loop and placed upon small rectangles of PB NN agar.

TABLE 2

Analysis of plasma membrane particles appearing in the cells under various conditions

	Negative area analyzed in cm^2	Numbers of particles measured	Average size of particles in Å and their S.D.
Immediately fixed control aggregates*	291.6	511	62 ± 14**
Mechanically disturbed aggregates	217.1 288.0	849 519	61 ± 11 90 ± 22
Control late aggregates	345.6	994	101 ± 27
20 µg/2 µl Con A added to aggregates	342.6	1,323	70 ± 16
1×10^{-3} M cyclic AMP on dispersed aggregates	327.7	1,130	97 ± 22

*Aldrich and Gregg (1973).

**The difference in particle sizes between the immediately fixed control aggregates (62 Å) and the smaller particles among the mechanically disturbed pair (61 Å) was not considered to be statistically significant ($p < 0.2$). The differences between the immediately fixed control aggregates and the four remaining aggregate preparations were all considered to be statistically significant ($p < 0.001$ in each instance).

Approximately 2 μl of Con A solution (10 mg/ml) was added to each aggregating stream which was sufficient to cover the entire surface area. The cells were exposed to the Con A by a gentle mixing process using a fine-tipped glass rod. Control preparations were identical in every respect with the exception that they were treated only with CAB.

Control aggregating streams required 4 hours to form late aggregates. The volume and concentration of Con A was carefully selected to effect a 4-5-hour morphogenetic delay in the aggregates. Consequently during the experimental period further development of the Con A treated streams was inhibited. At the end of 4-5-hour period each preparation treated with Con A or CAB was transferred with a hair loop into a tiny depression in the agar block to prevent its loss during the fixation and embedding process (Gregg, 1971). The control preparations upon thin-sectioning and examination with the electron microscope were shown to have synthesized PV typical of prespore cells. No evidence of prespore cell differentiation could be detected in the delayed aggregating streams. The Con A inhibited aggregates following the period of delay gradually recovered and formed mature sorocarps.

d. Con A effects on plasma membrane structure

Aggregating streams delayed for 4 hours with Con A and control aggregates exposed only to CAB were freeze-fractured. The control preparations which had formed late aggregates within 4 hours exhibited plasma membrane particles averaging 101 Å in diameter. The membranes of the Con A inhibited cells contained particles averaging only 70 Å (Table 2).

e. Effect of mechanical disruption on prespore cell differentiation

The aggregating streams were disrupted with a tiny glass rod every 20 minutes for 5 hours to prevent migrating pseudoplasmodium formation. Although this procedure did not disperse the cells many cell contacts may have been broken momentarily. This experiment was conducted to determine if cell differentiation will occur in spite of the periodically disrupted cell contacts which prevent the attainment of normal polarized late aggregates. Aggregating streams which were left undisturbed for a 5-hour period served as controls.

The disrupted aggregates examined by thin-section electron microscopy were devoid of PV although control late aggregates had differentiated prespore cells.

f. Effect of mechanical disruption on plasma membrane structure

The aggregating streams were prepared for freeze-fracture studies as described above under "e."

The freeze-fractured disrupted aggregates were found to have plasma membranes in two states of development. Approximately 80-90% of the cells exhibited membrane particles having an average size of 61 Å in diameter which is typical of the aggregation stage. The other 10-20% of the cells had much larger membrane particles averaging 90 Å which approaches the size of those found in prespore cells (Table 2). The undisturbed controls formed late aggregates within 4 or 5 hours which exhibited large particles averaging 101 Å in diameter.

Early Culminates

The small cylinders of agar bearing the beginning culminates were positioned such that the long axis of the slime mold was horizontal (Figure 5). This position prevented the drops of Con A solution (20 mg/ml) from touching the basal disc and the agar base which would result in a loss of the solution. The volume of Con A applied was approximately that of the volume of the prespore mass. By restricting the volume of Con A, which was applied with a fine-tipped glass pipette to the prespore area, the majority of the prestalk cells did not appear to be affected. The controls consisted of early culminates which had distilled water or CAB applied to the prespore area.

a. Effect of Con A, distilled water and CAB on culmination

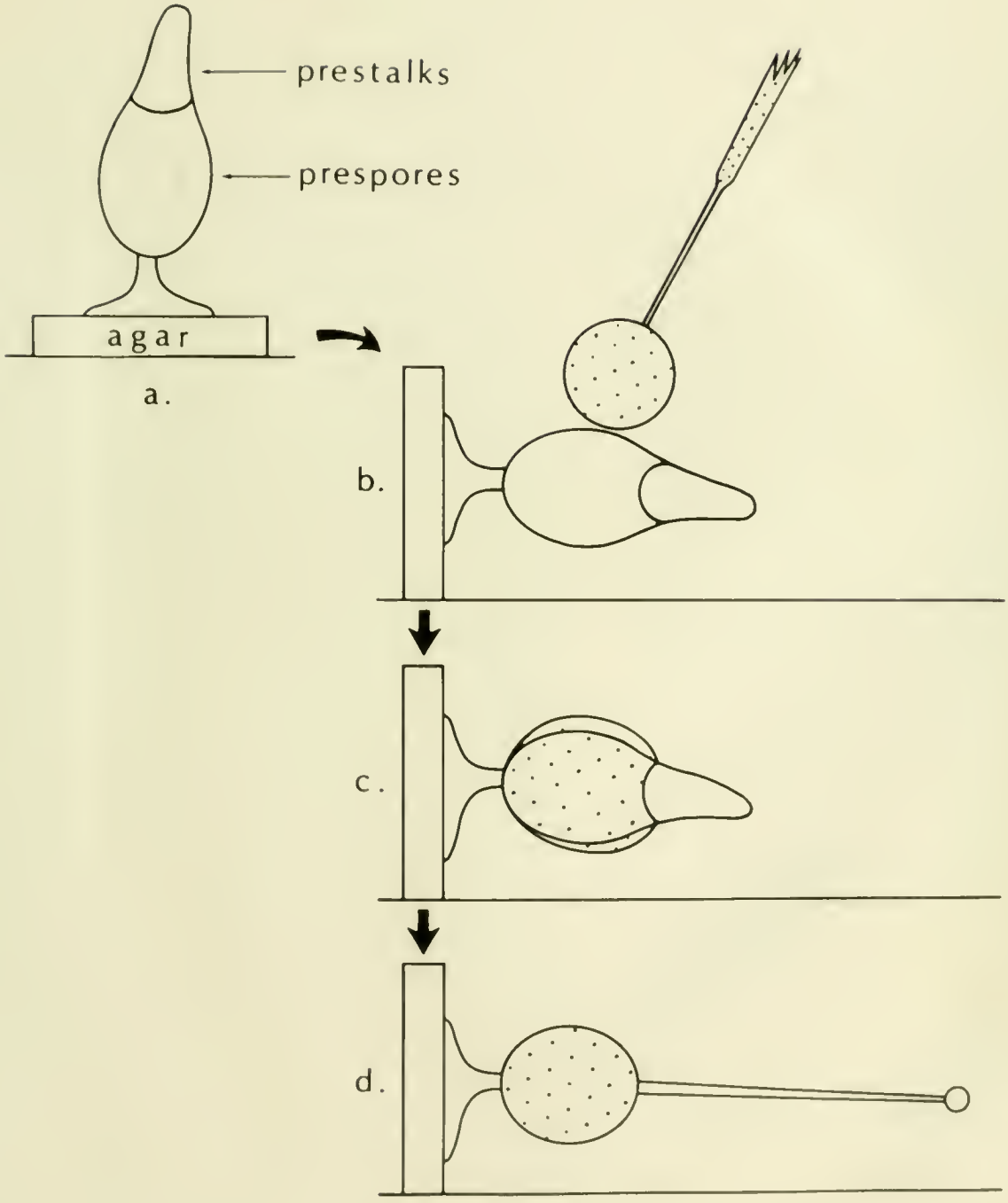
Beginning culminates in which the prespore area only was exposed to any one of the three agents formed fruiting bodies of the type illustrated in Figure 5. The prespore cells remained at the original level they had attained at the time the agent was applied. A group of cells composed primarily of prestalk cells but possibly including a small number of prespore cells continued to culminate. This resulted in a mature stalk bearing a small mass of normal mature spores at the apex.

b. Effect of Con A on mature spore differentiation

The cells on the peripheral layers of the prespore mass having the greatest exposure to Con A were devoid of PV and contained large autophagic vacuoles. Although no cell walls had developed, a layer of amorphous electron-dense material surrounded the cells which were abnormally irregular in shape. The mitochondria retained the form

Figure 5. Application of Con A or control solutions to the prespore area of early culminates.

a. early culminate on an agar base.
b. early culminate tipped to 90° angle for exposure to small volumes of reagents in the prespore area
c. prespore area exposed to reagents
d. type of mature sorocarp produced following exposure to reagents. (Apex composed of small mass of normal mature spores in both Con A and control preparations.)


prestalks
prespores
agar
a.
b.
c.
d.

normally observed in prespore cells (Figures 6, 7). The mitochondria in the remaining cells of the prespore mass were essentially typical of those found in normal mature spores. However, the PV were absent and cell walls failed to appear (Figure 8).

All of the cells composing the prespore mass upon exposure to distilled water or CAB differentiated into normal spores, lacking PV and exhibiting a cell wall (Figures 9, 10). Occasionally a few spores exhibited empty spaces in the cytoplasm which may have resulted from a loss of glycogen during the preparation for electron microscopy.

Figure 6. Cell from the peripheral region of the prespore mass after exposure to Con A, X19,566. Note the defective cell wall composed of an amorphous layer of electron-dense substance surrounding the cell surface.

AV - autophagic vacuole
M - mitochondrion, typical form found in prespore cells
N - nucleus

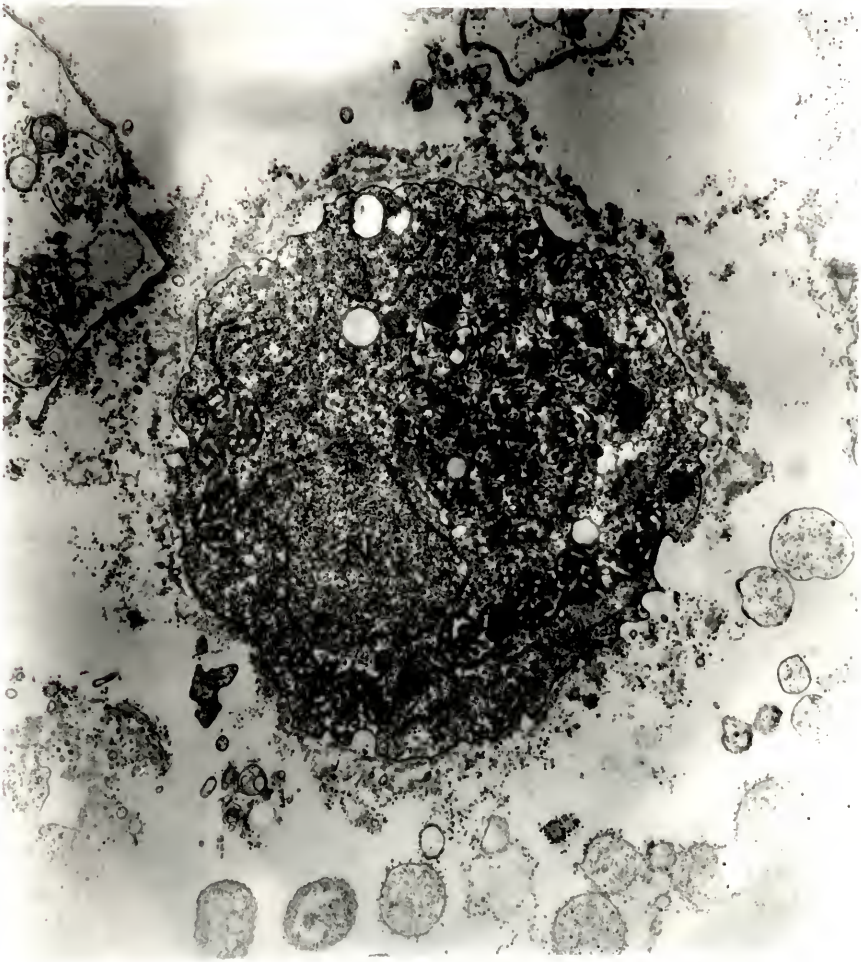


Figure 7. Cell from the mid-prespore region after exposure to Con A, X26,088. Note the layer of electron-dense substance composing the defective cell wall and the irregular cytoplasmic spaces which may have contained deposits of glycogen.

M - mitochondrion, typical form found in prespore cells

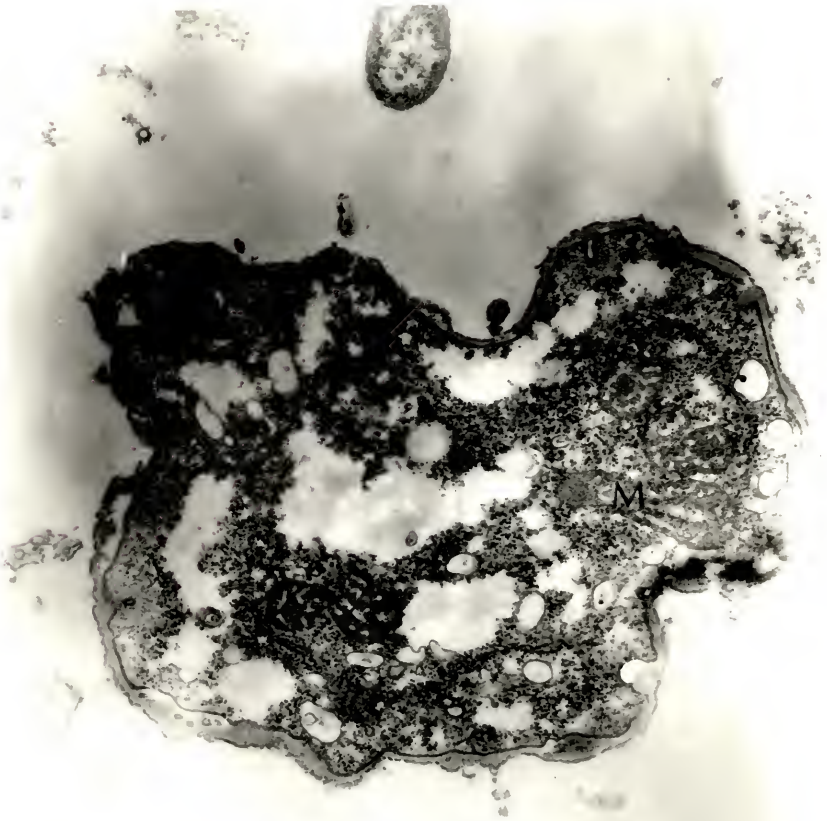
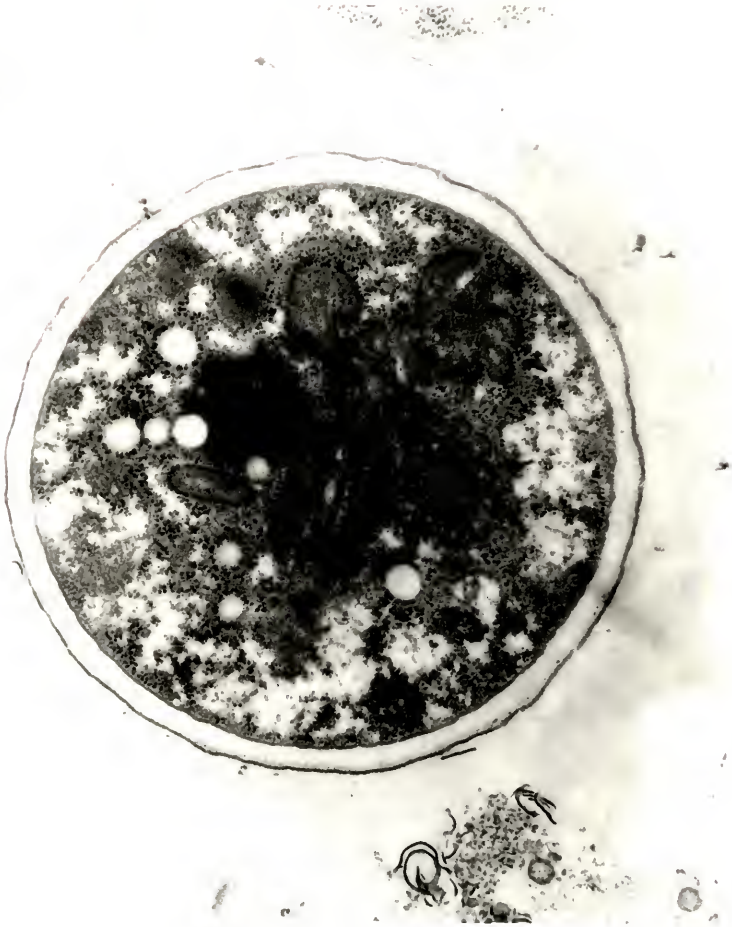

M

Figure 8. Cell from the central region of the prespore mass after exposure to Con A, X26,088. Note that the cell wall is atypical in thickness and associated with the cell surface by a layer of electron-dense substance. Arrows denote mitochondria essentially similar to those observed in controls.

Figure 9. Cell from the prespore region after exposure to distilled water exhibiting normal cell wall, X26,088. Arrows denote mitochondria typical of normal mature spores.

N - nucleus

Figure 10. Cell from the prespore region after exposure to CAB exhibiting normal cell wall and the irregular cytoplasmic spaces which may have contained deposits of glycogen, X34,784. Arrows denote mitochondria typical of normal mature spores.



DISCUSSION

Cyclic AMP in Prespore Differentiation

Cell differentiation in *Dictyostelium* depends upon cell association. Aggregation of the cells in response to the chemotactic stimuli from cyclic AMP results in the necessary contacts (Konijn *et al.*, 1967). The close association between the cells provides the opportunity for a potential accumulation or exchange of critical metabolites as well as interaction between components of the plasma membranes. The identity of metabolites possibly involved in effecting cell differentiation are obscure, with the exception of the report by Bonner (1970) that cyclic AMP induces mature stalk cell formation. A major increase in the average size of the plasma membrane particles has been correlated with the differentiation of the prespore cells (Aldrich and Gregg, 1973). The particles are the only ultrastructural components in the membrane known to change during this period although their precise role in development has not been established.

Gregg and Nesom (1973) determined that the synthesis or assembly of the large plasma membrane particles could be induced in vegetative myxamoebae within 2 hours by exposure to cyclic AMP. Myxamoebae from aggregating streams dispersed as single cells for 6 hours upon agar containing cyclic AMP synthesized particles averaging 97 Å in size. This represented a 1.6X increase above the immediately fixed control preparations at 62 Å (Table 2). Neither vegetative myxamoebae nor

aggregating streams were found to contain PV (Gregg and Badman, 1970). Upon thin-sectioning and examining dispersed vegetative and aggregating stage cells exposed to cyclic AMP it was determined that PV had not formed indicating that the cells had failed to differentiate into prespores. Thus, the synthesis of large particles may occur independently of PV formation and prior to cell differentiation. Occasionally a few small aggregates formed on the cyclic AMP agar. These cells had synthesized large particles and also PV. This emphasizes the necessity of cell contact in promoting cell differentiation. Preparations of aggregating streams left undisturbed during the 6-hour period as controls had formed late aggregates containing PV and particles averaging 101 Å.

Cyclic AMP also induces adhesiveness between the cells (Konijn *et al.*, 1968) and simultaneously causes an efflux of Ca^{++} (Chi and Francis, 1971) which may also be involved in the appearance of large membrane particles (Gregg and Nesom, 1973). The plasma membrane in *D. discoideum* is approximately 70 Å in thickness. It is conceivable that the larger particles and their associated carbohydrate components project through the membrane and are exposed at the cell surface. This suggests that cell adhesion may be effected by the interaction between the projecting particles. Under these circumstances movement or rearrangement of the particles within a fluid plasma membrane might occur. As a result of the shifting particles stimuli may be transmitted to intracellular components which in turn initiate the process of differentiation.

During normal development of *Dictyostelium* it is probable that following aggregation the concentration of cyclic AMP increases within

the cell mass and initiates large particle formation. A period of cell interactions possibly mediated through the particles results in prespore cell differentiation. The necessity of the cells to maintain contact in order to differentiate was determined by mechanically disrupting the aggregate every 20 minutes for 5 hours. The greater proportion of the cells examined by freeze-fracturing had not synthesized large particles suggesting that cyclic AMP secretion may have been inhibited. A small proportion of the cells exhibited particles averaging 90 Å which approaches the average size of those found in prespore cells. Neither group of cells had differentiated into prespore cells as indicated by the absence of PV. The controls had attained the late aggregate stage in 5 hours and exhibited both large membrane particles and PV. Consequently it appears that a certain period of continuous contact between the cells is essential in cell differentiation.

Con A in Prespore Differentiation

Con A binds to vegetative myxamoebae cell surfaces and quickly effects agglutination under appropriate conditions. If the cells are exposed to Con A in agar the rate of aggregation is inhibited as well as the number of mature sorocarps which ultimately appear (Table 1). The ability of Con A to bind to cell surfaces provided a tool to elucidate the nature and role of cell contacts in differentiation. Groups of cells isolated from aggregating streams require approximately 4 or 5 hours to attain the late aggregate stage. If such preparations are exposed to Con A the cells are held in contact to the degree

allowed by the Con A bonded to their plasma membranes. These aggregating streams were delayed throughout the period required for the controls to form late aggregates. Electron microscopy of the delayed preparations revealed that membrane particles had formed which were only 1.1X larger than the immediately fixed controls. However, the synthesis of particles of this magnitude was not followed by PV formation. The controls forming late aggregates contained PV and particles 1.6X greater than the immediately fixed controls (Table 2).

The chemotactic response to cyclic AMP also depends upon a membrane bound phosphodiesterase (PDE). PDE increases sharply in activity just prior to aggregation but returns to lower levels shortly after this morphogenetic event is initiated (Malchow *et al.*, 1972). The failure of the aggregates in differentiating prespore cells may be attributed to the ability of Con A to cause the synthesis of excessive amounts of PDE in vegetative myxamoebae prior to its customary appearance at aggregation (Gillette and Filosa, 1973). Observations of other systems suggest that alterations in the activity of PDE or adenyl cyclase may result from the clustering of Con A glycoprotein receptors at the cell surface (Edelman *et al.*, 1972). Guérin *et al.* (1974) have determined that Con A induced agglutination of murine plasmocytoma cells was accompanied by the clumping of intramembranous particles. The Con A binding sites are not considered to be associated with the particles in either plasmocytoma or in *E. histolytica* membranes (Martínez-Palomo *et al.*, 1974).

The existence of cyclic AMP binding sites on the cell surface of *D. discoideum* has been reported by Moens and Konijn (1974). The possibility exists that Con A masks these sites and prevents cyclic

AMP from bonding to the membrane. It is conceivable that PDE activity may also be increased by Con A among the cells in an aggregating stream as well as in vegetative myxamoebae. Both PDE and masked cyclic AMP binding sites could have the effect of reducing the concentration of cyclic AMP available at the cell surfaces. Consequently normal morphogenetic movements could be delayed as well as the assembly of large membrane particles which depend upon the presence of cyclic AMP.

Con A in Culmination and Mature Spore Differentiation

By the late aggregation stage large plasma membrane particles have been synthesized and both prestalk and prespore cells have differentiated. If a group of prespore cells are isolated from a migrating pseudoplasmodium a certain proportion will redifferentiate into prestalk cells devoid of PV (Gregg and Badman, 1970). If the prespores are isolated as single cells neither redifferentiation into prestalk nor differentiation into mature spores can occur (Gregg, 1971).

The differentiation of the prespore cells into mature spores begins during the mid-point of the culmination process (Figure 1i). An early culminate which has formed a basal disc and whose prespore cell mass has barely cleared the agar substratum will not have differentiated mature spores (Figures 1h, 5a). Early culminates disorganized periodically for 6 hours with a glass needle cannot differentiate mature spores (Gregg and Badman, 1970). Obviously cell contact is required for either redifferentiation or differentiation at various points in the development of *Dictyostelium*.

During the normal transition of a prespore into a mature spore, PV disappear from the cytoplasm and are considered to be incorporated into the spore wall (Hohl and Hamamoto, 1969). Although small volumes of distilled water, CAB and Con A affected the morphogenetic process of culmination (Figure 5) only Con A prevented normal spore differentiation. The major defect observed was in the formation of the spore wall. PV also were not found among the defective spores induced by Con A. This suggests that Con A is not bound to the sites involved in the cell interactions which may effect the synthesis or loss of PV. However, the presence of bound and free Con A at the surface of the prespores produced irregular shaped spores surrounded by a layer of amorphous electron-dense material in place of a cell wall (Figures 6, 7, 8). This aberrant wall may have resulted from Con A agglutinating and interferring with the PV and other constituents which were liberated at the cell surface in the course of spore differentiation. The defective spores retained mitochondria having the typical form of those in prespore cells (Figures 6, 7). This may be a consequence of the failure of the spore to attain its normal morphogenetic shape resulting in further effects upon the morphology of these cytoplasmic organelles.

Role of Cell Contacts and Interaction in Differentiation

The induction of plasma membrane particles by cyclic AMP averaging approximately 100 Å in diameter is always associated with cell differentiation in *Dictyostelium*. Although the particles may be synthesized among isolated single vegetative or aggregating cells this event is not simultaneously accompanied by prespore cell differentiation. If aggregates are mechanically disrupted periodically for 5 hours a few cells exhibit large particles but PV formation does not occur. Obviously

several hours of continuous contact between the cell surfaces is necessary to effect prespore cell differentiation. Upon aggregation in response to cyclic AMP the cells become adhesive (Konijn *et al.*, 1968). The contact sites active in the adhesive process may be inhibited by specific univalent antiserum. Although random cell movement continues migrating pseudoplasmodium formation is prevented (Beug *et al.*, 1970). Univalent Con A, however, does not interfere with normal morphogenesis in *Dictyostelium* (Weeks and Weeks, 1975). The possibility that *Dictyostelium* adhesive sites are associated with the membrane particles has not been established. The relative effects of univalent Con A and antiserum suggest that the adhesive sites could be associated with the particles considering that Con A fails to bind to those membrane constituents (Martínez-Palomo *et al.*, 1974). Divalent Con A delays morphogenetic movements and prevents cell differentiation probably by indirectly effecting a reduction in the cyclic AMP necessary in particle formation. Thus, any cellular interactions mediated through contacts among these membrane particles could not occur.

During the process of culmination prespore cells bearing large membrane particles differentiate into mature spores. The PV distributed in the prespore cytoplasm break down and become part of the spore wall (Hohl and Hamamoto, 1969). Exposure of the prespores to Con A just prior to spore differentiation does not hinder the disappearance of the PV. Since cell contact is essential in spore formation (Gregg and Badman, 1970) the Con A evidently does not mask nor affect the sites necessary in effecting PV loss. However, Con A does act at the cell

surface causing defective cell walls to appear as well as abnormally formed spores containing atypical mitochondria.

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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